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(54) Title: THE MICROBIAL PREPARATION & METHOD FOR PREVENTING AND CURING THE BACTERIAL WILT THE PLANT AND ITS USE

(54) 发明名称: 防治植物细菌性青枯病的微生物制剂和方法及其用途

(57) Abstract: The present invention relates to a kind of microbial preparation, which contains Paenibacillus polymyxa or its Back-extraction. The said microbial preparation can be used for preventing and curing the plant bacterial wilt and seedling blight at the seedling stay. damping-off, fusarium wilt of cucumber, Tomato fusarium wilt, tobacco brown spot, soybean root rot and so on, it also has strong growth stimulatory activity for plant.

(57) 摘要

本发明提供了一种农用微生物制剂,该微生物制剂含有多粘类芽孢杆菌或利用 该菌培养而得的发酵清液。本发明的微生物制剂不仅可用来防治植物细菌性青枯病, 而且还可用来防治植物苗期立枯病、猝倒病,以及黄瓜枯萎病、番茄枯萎病、茄子 枯萎病、烟草赤星病、大豆根腐病,并且对植物具有明显的促进生长作用。

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防治植物细菌性青枯病的微生物制剂和方法及其用途

技术领域

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本发明涉及微生物领域。具体地说,本发明涉及一种防治青枯病的微生物制剂及其方法和用途。

背景技术

青枯病是由青枯劳尔氏菌(Ralstonia Solanacearum)引起的一类世界性的土传植物病害,在热带和亚热带地区发病普遍,危害严重。青枯病菌的寄主范围很广,在植物的根内和土壤中有很强的存活能力,可侵染 44 个科 300 多种植物。

对于植物青枯病的防治,多年来国内外均予以高度重视。然而迄今为止,抗青枯病的作物品种很少,抗性低且抗性极易丧失,这主要是由于青枯病病原菌群的复杂性和作物品种本身等因素所致。此外,抗病品种品质差、产量低,难以推广。其它方法,如嫁接虽获得成功,但由于其技术难度高,难以大面积推广;农业措施如水旱轮作等受地域条件限制,也难以大面积推广。农药如农用链霉素、铜试剂(如77%可杀得)等虽然在田间表现出一定的防效,但由于这些农药均不是防治青枯病的农药,因而田间防效较差,不稳定,且病原菌易对这些农药产生抗药性。因此,目前尚无防治植物青枯病的有效药剂。

20 青枯病的发病机理大致如下:存在于土壤中的青枯病病原菌在寄主作物的整个生长期均可通过植物根系侵染植物内部并潜伏,在合适条件(如高温、高湿)下潜伏在植物体内的青枯病病原菌便大量繁殖,堵塞植物的维管束发展成为病害。针对上述特点,可采用从作物苗期开始防止病原菌从根系侵入和潜伏并阻止潜伏病原菌发展成为病害的防治策略。

从80年代初开始,国内外在采用无致病力的青枯假单胞菌菌株防治青枯病的研究和应用方面开展了许多工作,发表了大量的文章。但研究工作几乎都停留在温室阶段,至今大田试验尚未取得成功。同时,无致病力的青枯假单胞菌菌株在自然条件下还可能存在变异,因此,其应用潜力不大。

另外,细菌素产生菌株(ABPS)、荧光假单胞菌(Pseudomonas fluorescens)、颍壳假单胞菌(Pseudomonas glumae)及其变种、洋葱假单胞菌(Pseudomonas cepacia B5)、青枯假单胞菌(Pseudomonas solanacearum)的变种、芽胞杆菌(Bacillus spp.B33 和 B36)以及泡囊丛枝菌根真菌(VAM)等生防菌株也被先后用来防治植物青枯病。然而,这些菌株也仅仅在温室或田间苗期有效果,而在定植 40 天后几乎没有防治效果。

因此,本领域中迫切需要能够有效防治植物青枯病的新的微生物制剂和方法。

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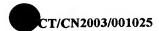
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本发明的目的是为了提供能够防治植物青枯病的新的微生物制剂和方法。

发明者通过研究从江西南昌郊区土壤中筛选分离获得的一株多粘类芽孢杆菌,该菌株的活菌、发酵液或发酵清液在大田试验中成功地防治了番茄、辣椒、茄子和烟草青枯病,从而完成了本发明。该菌株已经于 2002 年 10 月 31 日保藏于中国微生物菌种保藏管理委员会普通微生物中心(CGMCC),保藏号为 CGMCC No. 0829。

因此,本发明第一方面提供了一种多粘类芽孢杆菌(Paenibacillus polymyxa) HY96-2, 其保藏号为 CGMCC No. 0829。

本发明第二方面提供了一种农用微生物制剂,该微生物制剂含有多粘类芽孢杆菌活菌或利用该菌培养而得的发酵清液。在一个具体实施方案中,该多粘类芽孢杆菌是 CGMCC No. 0829。

在一个较佳的实施方案中,所述微生物制剂含有利用该菌培养而得的发酵液,如含有多粘类芽孢杆菌 CGMCC No. 0829 的活菌以及利用该菌培养而得的发酵清液。

本发明所用的术语"发酵液"、"活菌"、"发酵清液"具有本领域技术人员通常熟知且承认的含义。所述发酵液可通过在适合生长的条件下培养本发明的多粘类芽孢杆菌 CGMCC No. 0829(即下文所称的"生防菌[biocontrol agent]HY96-2"),使其生长至一定的细菌浓度来获得;所述的活菌是指通过生物法培养生防菌而得到的具有生活能力的菌体;所述的发酵清液是指将发酵液通过分离去除其中的菌体而得到。

用于培养本发明菌株的培养基中的营养源没有特别的限制。本领域技术人员可以根据公知的技术来选择合适的碳源、氮源和其它营养源。例如,碳源可以是淀粉、糊精、甘油、葡萄糖、蔗糖、肌醇、甘露醇等。氮源可以是胨、大豆粉、蛋白粉、肉膏、米糖、麦皮、酵母粉、玉米浆、铵盐以及其它有机或无机含氮化合物。另外,培养基中还可适当加入一些无机盐类,如氯化钠、磷酸盐如磷酸氢二钾和磷酸二氢钾、硫酸铵、硫酸锰、硫酸镁、碳酸钙等金属盐。通常可采用各种已知的常规培养基,如 LB 琼脂培养基、营养琼脂培养基、葡萄糖酵母膏琼脂培养基和牛肉浸汁琼脂培养基等。下文实施例中给出了一个最适培养基的配方。然而,本领域技术人员应当理解,本发明并不局限于本文中列举的这些具体培养基配方。

培养本发明菌株时的温度、pH、通气比、罐压、转速等条件没有特别严格的限制,只要该条件适合所述菌的生长即可。在培养时可采用豆油、泡敌等消泡剂进行消泡。在一些较佳的实施方案中,pH 宜控制在 5.5-7.5 之间。培养温度宜在 25-35 $^{\circ}$ 之间。培养时间通常在 12 小时至 72 小时之间。最终的菌浓度通常可高达 1×10^{11} CFU/ml 至 1×10^{12} CFU/ml。然而,上述列举的这些参数只是实现本发明目的的较佳方案。因此,本领域技术人员在上述范围以外选择合适的培养条件也能获得本发明的活菌体、发酵清液、发酵液。

本发明的微生物制剂例如可以直接以发酵液形式施用,也可将其适当稀释(例如

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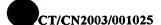
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稀释 10 倍、100 倍、1000 倍或更高)以稀释液形式施用,还可用本领域常规技术对所述发酵液进行分离提取。获得的活菌体、发酵清液以及从中获得的提取物也可直接施用。微生物制剂中还可含有不影响发酵液防治青枯病效果的其它物质。例如,为了便于长期贮存,可将发酵原液或其稀释液与合适的载体混合,然后适当干燥,制成载体形式的微生物制剂。这些载体对本发明的活菌体、发酵清液以及发酵液防治植物青枯病效果无影响。因此,在一个较佳的实施方案中,所述微生物制剂还含有选自稻壳粉、玉米秸粉、草炭土、轻质碳酸钙、滑石粉、凹凸棒土和/或硅藻土的载体及其混合物,其中较佳的载体是稻壳粉、凹凸棒土或玉米秸粉。这些载体均可市售购得。载体宣事先加工研磨成粒度在 10-200 目之间。载体与含活菌的菌悬液、发酵清液或发酵液宜以 1: 0.1 至 1:10 的重量比例,更佳的为 1: 0.2 至 1: 5 的重量比例混合。上述干燥步骤可以采用本领域中的常规技术,例如但不局限于,自然干燥法、真空干燥法、气流干燥和沸腾床干燥法等。为了能使多粘类芽孢杆菌尽可能保持较高的活性和较长的贮存期,有机载体形式的微生物制剂的含水量宜控制在 3-16%(重量)之间,更佳的为 7-16%(重量)之间,无机载体形式的微生物制剂的含水量宜控制在 3-6%(重量)之间。

本发明另一方面提供了一种防治植物细菌性青枯病的方法,该方法包括将本发明上述微生物制剂施加到患青枯病植物的根部上的步骤。

上述将微生物制剂施加到植物根部上的方法是本领域中的常规技术,例如可以 是在播种时浸种,移栽前将植物根部浸在发酵液或其稀释液中,或者直接将发酵液 或稀释液泼浇在苗床上,可定植时进行灌根,也可在植物生长过程中灌根。若微生 物制剂是以载体形式保存的,则可在临用前用水稀释后再进行施加。

本领域技术人员无需经过过多试验即可确定本发明的最适施药剂量。例如,当以稻壳粉作为载体时,较佳的施药剂量在平均每亩(667平方米)1.5~4.5千克范围内。

在一个较佳的实施方案中,本发明方法可用来防治番茄、辣椒、茄子和烟草作物的青枯病。如下文试验结果表明,本发明的微生物制剂可用于防治番茄、辣椒和茄子等作物青枯病, 收获后期(有的对照发病率高达 97%)的田间防效可达 70~85%。

本发明者还发现,本发明的微生物制剂也可用来防治植物苗期立枯病、猝倒病 以及番茄枯萎病、茄子枯萎病、黄瓜枯萎病,烟草赤星病和由镰刀菌引起的大豆根 腐病等真菌性病害。

因此,本发明还有一个方面涉及本发明所述微生物制剂用于防治植物苗期立枯病、猝倒病,以及番茄枯萎病、茄子枯萎病、黄瓜枯萎病、烟草赤星病、大豆根腐病的用途。

本发明者还发现,在植物不发青枯病时,本发明的微生物制剂具有明显的促进植物生长、提高产量的作用(例如提高番茄产量 27.5%);而且发明的微生物制剂对其它作物,如菠菜、苋菜、豇豆、黑麦草等等,也具有明显的促进生长、提高产量,



增产高达 18~25%。

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因此,本发明还有一个方面涉及微生物制剂在促进植物生长、提高植物产量上的用途。

本发明的微生物制剂具有生防作用强的优点,作为生物农药应用的潜力很大。 具体表现在:第一,它能有效防治番茄、辣椒、茄子和烟草等作物的青枯病,推迟 发病。第二,在植物发病的后期,特别是植物收获后期仍具有很高的防治效果,最 高可达 85%以上,这一防效在其他人的研究中未见报道。第三,该生物农药制剂还 能显著提高作物的产量,除对这些青枯病发病作物生长有明显的促进作用外,对其 它植物的生长也具有明显的促进作用。第四,该微生物制剂还可防治植物苗期立枯 病、猝倒病,番茄枯萎病,茄子枯萎病,黄瓜枯萎病,烟草赤星病以及由镰刀菌引 起的大豆根腐病等真菌性病害。

本发明的其它优点和目的可从下文进一步详细描述中清楚地得知。

保藏信息

本发明菌株 HY96-2 已经于 2002 年 10 月 31 日保藏于中国微生物菌种保藏管理委员会普通微生物中心(CGMCC,中国,北京),保藏号为 CGMCC No. 0829。

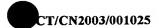
实施例 1 菌株 HY96-2 的分离筛选

本实施例以青枯劳尔氏菌株(Ralstonia solanacearum)1号生理小种 Tb 和 2号生 20 理小种 Tt、蔬菜立枯病菌、烟草赤星病菌、稻瘟病菌、黄瓜枯萎镰刀菌以及大豆根腐镰刀菌作为指示菌株。

在南昌郊区发生程度不同的青枯病田采集番茄健株和病株,连同根系周围的部分土壤装入干净的塑料袋内密封,带回室内尽快分离。将采集的样本分为以下 3 部分:根围,用力抖动植株,抖下的土壤为根围部分;根际:抖动后较牢固地沾附于根表的土壤,用水洗下,为根际部分。根表:经洗涤的根剪成小段,与石英砂和水混合,充分振荡,洗下部分为根表部分。

将上述 3 部分分别进行稀释法分离,培养基为改进的酵母浸膏平板(葡萄糖: 1.0%; 酵母浸膏: 0.5%; KH_2PO_4 : 0.05%; $MgSO_4$: 0.05%; 琼脂粉: $1.5\sim1.6\%$; pH: $7.2\sim7.4$; 121 C 高压灭菌 25 分钟),在每个平板上再加入适量的青枯菌 Tb 和 Tt 悬液,置 $28\sim30$ C 温箱中培养,将形态各异的单个细菌菌落挑出,再划线纯化。保存于斜面培养基上待用。

然后用以下方法确定拮抗菌。第一种方法是:将分离物点接于改进的酵母浸膏平板上,30℃培养 48 小时,以氯仿熏蒸杀死,涂布青枯菌 Tb 和 Tt 悬液(108cfu/m1),继续培养 12~24 小时,观察菌落周围有无抑菌圈及其大小,记录抑菌圈大小,统计拮抗菌占总分离菌数的比例。纯化所有表现拮抗作用的菌株,留待下一步试验。



第二种方法是:将青枯菌 Tb 和 Tt 悬液(10⁸cfu/ml)1ml 加入灭菌培养皿中,再加入 50℃左右的 15ml 培养基,摇匀。冷却后,点接分离到的细菌菌株,每皿接 5个分离菌,置 30℃培养 12~24 小时,观察菌落周围有无抑菌圈及其大小,记录抑菌圈大小,统计拮抗菌占总分离菌数的比例。纯化所有表现拮抗作用的菌株,留待下一步试验。

然后,测定所获得的拮抗菌转移 10 次后的拮抗能力,选取仍具有拮抗能力的菌株保存。将上述获得的菌株对其他几种病原菌的拮抗能力进行测定,采用平板对峙培养法(参见《植病研究方法》,方中达编,1979,农业出版社)。

结果与分析

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本实施例共采集样本 40 个,其中重病田(病株率在 50%以上)健株、病株和轻病田(病株率在 20%以下)健株、病株各 10 株。初次筛选得到拮抗细菌 206 株,其中来自重病田健株、病株和轻病田健株、病株的各为 89、54 和 35、28 株;根围、根际和根表各为 122,53 和 31 株。在这 206 株菌中,通过上述两种拮抗试验,获得对青枯病具有一定拮抗作用的菌株 98 株。

98 个拮抗菌株于改良的酵母浸膏斜面转移 10 次,每次间隔 6 天,以青枯菌 Tb 和 Tt 重新测定拮抗能力,仅有 49 株保持原拮抗能力,其余菌株全部丧失。按分离部位来看,49 个菌株中有 21 个来自根表;根围和根际各有 12 个和 16 个。说明根表菌株的分离频率高于根围和根际。下表 1 中列出部分拮抗作用表现较好的菌株。

表 1. 分离的部分菌株对青枯菌的抑菌情况

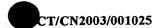
	农1. 万两时即为图外对自己图出对中国问题										
土样	分离菌株	抑菌圈平均直径(mm)			土样	分离菌株	抑菌圈平均直径(mm)			ım)	
编号		Т	b	Ti	:	编号		Т	ъ	Tt	
		方法 1	方法 2	方法 1	方法 2			方法 1	方法2	方法1	方法 2
1-1	HH-3	20.0	18.5	17.0	16.6	3-1	HH-34	20.4	15.6	16.5	14.3
	HH-5	22.6	17.0	20.6	15.2		HH-42	24.7	17.8	20.6	18.2
1-2	HY-2	35.2	30.8	36.8	32.6	3-2	HY-3	30.5	25.7	32.5	30.4
	HY-14	30.4	29.2	25.5	25.8		HY-30	17.6	12.6	12.5	11.8
	HY-22	18.4	13.2	15.2	13.6	3-3	HF-22	15.8	12.2	17.6	15.3
2-2	DY-15	25.6	20.4	27.8	22.5	4-1	DH-16	12.5	10.5	14.2	12.9
2-3	DF-26	10.8	9.2	12.5	10.8		DH-18	21.9	16.8	21.2	18.8
						4-2	DY-21	16.7	13.6	18.6	16.7

使这些菌株再与蔬菜立枯丝核菌、烟草赤星病菌、稻瘟病菌、枯萎镰刀菌、大豆根腐镰刀菌进行对峙培养,结果如表 2 所示。

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农 2										
病原菌		抑菌带宽度(mm)								
	HY-2	HY-2 HH-3 HY-14 DY-15 H								
立枯丝核菌	25.6	20.8	26.8	15.8	17.3					
赤星病菌	28.5	14.5	18.2	16.2	18.8					
稻瘟病菌	22.4	22.3	20.5	18.5	22.6					
枯萎镰刀菌	32.1	12.5	13.5	15.3	24.2					
根腐镰刀菌	35.7	14.3	18.6	14.2	26.4					

表 2 拮抗菌株对植物真菌病害病原菌的抑菌作用结果

上述结果表明,由番茄根际获得的菌株 HY-2、HY-14、HY-3 除对植物青枯病的两个生理小种均具有明显的拮抗作用外,对其它几种病原菌也具有很好的拮抗作用。这表明 HY-2 等菌株拮抗作用强、抗菌谱广。将这些菌株命名为 HY96-2、HY96-14和 HY96-3。

实施例 2 HY96-2 菌株的鉴定

下面进一步对上述实施例 1 中获得的分离自南昌番茄根际土壤的 HY96-2 菌株进行鉴定。

染色:按本领域常规方法进行革兰氏染色和抗酸染色。

形态特征:于营养琼脂、牛肉浸汁琼脂培养基上 32℃培养 2 天,取菌体涂片,染色后用光学显微镜观察菌体形态,并用电子显微镜观察细胞的表面特征。

细胞壁化学分类: 用薄板层析法对菌体进行全细胞水解液的氨基酸及糖型分析。 培养特征: 在 LB 琼脂、营养琼脂、葡萄糖酵母膏琼脂和牛肉浸汁琼脂四种培养基 32℃培养 2-3 天后观察菌落形成及颜色。

生理生化特征: 参照《Bergey's Manual of Systematic Bacteriology》Vol. II 的方法和《常见细菌系统鉴定手册》进行。

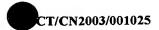
16S rDNA 序列分析: 按常规方法提取菌株总 DNA。采用通用引物进行 16S rDNA 的 PCR 扩增,PCR 产物纯化后直接用 Taq DyeDeoxy Terminator Cycle Sequencing Kit 测序,电泳及数据分析由 Applied Biosystems DNA Sequencer (model 377)自动进行。将所测定的 16S rDNA 序列 GenBank 数据库中相关种、属序列比较,以确定该菌株的分类地位。

实验结果:

- (1) 革兰氏染色结果表明, HY96-2 菌株为革兰氏阳性兼有阴性, 抗酸染色呈 25 阴性。
 - (2)形态特征:于32℃培养2天后,HY96-2 菌株的菌体为直或近直的杆状,在一个略膨大的孢囊内只含有一个椭圆形芽孢,并具有稀疏的周生鞭毛,能运动; 好氧生长,兼性厌氧,在营养琼脂上无可溶性色素。

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- (3) 细胞壁化学分析: HY96-2 菌株含有 meso-DAP(二氨基庚二酸)甘氨酸, 无特征性糖, 细胞壁Ⅱ型。
 - (4) 培养特征: HY96-2 培养特征见下表 3。

表	3.	HY96-2	菌株的培养特征
~~	\sim .		ET NICHA HOLLIA FE

培养基	菌落颜色及形状				
LB 琼脂	菌落乳脂色	湿润光滑			
营养琼脂	菌落杏仁白色	粘稠状			
葡萄糖酵母膏琼脂	菌落淡黄白色	有小突起,粘性			
牛肉汁琼脂	菌落灰白色	湿润光滑			

(5) HY96-2 菌株的生理生化特征: 见表 4。

表 4. HY96-2 菌株的生理生化特征

特征	结果	特征	
6.5%NaCl生长	-	淀粉水解	+
触酶反应	+	水解尿素	-
氧化酶反应	+	水解卵磷脂	-
硝酸盐还原	+	水解七叶灵	-
V-P 试验	+	利用糖产酸:	
吲哚反应	-	葡萄糖	+
H₂S 反应	-	L-阿拉伯糖	+
柠檬酸盐利用	-	L-鼠李糖	-
明胶液化	-	果糖	-
纤维素上生长	-	木糖	+
吐温 80	+	甘露醇	+
41℃生长	+	半乳糖	+
4℃生长	-	核糖	

(6) 16S rDNA 序列分析: 16S rDNA 序列分析结果表明, HY96-2 菌株属于类 芽孢杆菌属(Paenibacillus)。HY96-2 菌株与多粘类芽孢杆菌(Paenibacillus polymyxa) 序列同源性为 99%。

根据 16S rDNA 的序列分析结果,HY96-2 菌株属于类芽孢杆菌属;HY96-2 菌株革兰氏染色阳性兼有阴性,不抗酸,细胞呈杆状,形成芽孢,并有鞭毛,在一个孢子囊中只产生一个孢子,属于类芽孢杆菌属(Paenibacillus)。HY96-2 菌株的各项培养特征和生理生化特征均与多粘类芽孢杆菌相同。所以将 HY96-2 菌株鉴定为多粘类芽孢杆菌(Paenibacillus polymyxa)。该菌株已经于 2002 年 10 月 31 日保藏于中国微生物菌种保藏管理委员会普通微生物中心(CGMCC),保藏号为 CGMCC No. 0829。

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实施例 3 HY96-2 的发酵培养

1) 5L 全自动发酵罐培养

将 HY96-2 种子活化后进行菌种培养。先将淀粉糊化,再加到含酵母粉、蛋白粉、葡萄糖、MgSO₄、KH₂PO₄和 CaCO₃的培养基中,121℃灭菌 30 分钟。

用无菌水洗下茄子瓶中的菌体,接种到 5L 全自动发酵罐中。在通气比为 0.4~2: 1,转速 300~800rpm, 25~35℃的条件下,发酵时间 24~48 小时。采用平板计数法(参见《植病研究方法》,方中达编,1979,农业出版社)测得其最后菌浓度为 1.37×10¹²CFU/ml。

2) 50L 全自动发酵罐培养

种子活化后进行菌种培养。先将淀粉糊化,再加到上述 1)的培养基中。121℃ 灭菌 30 分钟。种子接种到 50L 全自动发酵罐中。在通气比为 0.4~2: 1,转速 300~800rpm,温度 25~35℃的条件下,发酵时间 24~48 小时。采用平板计数法测得其最后菌浓度为 2.09×10¹¹CFU/ml。

3) 1 吨发酵罐培养

在 5L 全自动发酵罐上进行种子培养,方法如上文 1)所述。

然后进行培养基灭菌。先将淀粉糊化,再加到含葡萄糖、酵母粉、蛋白粉、 $MgSO_4$ 、 KH_2PO_4 和 $CaCO_3$ 的培养基中。121 C 灭菌 35 分钟。将在 5L 全自动发酵罐中培养的种子接种到 1 吨发酵罐中。在通气比为 0.4~1: 1,转速 100~350rpm,25~35 C 的条件下发酵 24~48 小时。采用平板计数法测得其最后菌浓度为 1.02~10 CFU/ml。

实施例 4 载体形式的微生物制剂的制备

1)以稻壳粉为制剂载体

将稻壳粉进行加工,使其粒度在 10-100 目之间。将发酵液与载体稻壳粉混配 (0.2~5:1(重量比))后,搅拌,分别采用自然干燥法、真空干燥法和沸腾床干燥法进行干燥。获得含水量分别为 14%、13.2%和 14.3%的三种制剂。

2) 以凹凸棒土为制剂载体:

将凹凸棒土进行加工,使其粒度小于 44 微米。对发酵液离心分离,过滤获得活菌和发酵清液。清洗活菌数次,然后使其悬浮于与最初发酵液体积大致相同的水中。将所得活菌悬液与凹凸棒土混配(1:3(重量))后,搅拌,分别采用自然干燥法、真空干燥法和沸腾床干燥法进行干燥。使三种制剂含水量分别为 4.5%、4.8%和 4.0%。

3) 以玉米秸粉为制剂载体:

将玉米秸粉进行加工,使其粒度在20-40目之间。将上述2)中分离获得的发酵清液与玉米秸粉混配(3:1(重量))后,搅拌,分别采用自然干燥法、真空干燥法和沸腾

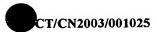
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床干燥法进行干燥。使三种制剂含水量分别为 14.1%、14.8%和 13.8%。

用下述方法鉴别微生物制剂: 称取样品 1g, 置于三角瓶中, 加 10ml 灭菌水, 于旋转式摇床上以 150rpm 充分振荡温育 1 小时, 立即取 1ml 悬浮液加入一支盛有 9ml 灭菌水的试管中, 用接种环蘸取悬浮液在酵母浸膏培养基平板上划线, 立即将 平板置于 30℃恒温箱中培养, 48 小时至 72 小时之内观察菌落形态。

根据多粘类芽孢菌的培养性状鉴别。其培养性状为:在酵母浸膏培养基平板上生长良好,样品中的细菌菌落颜色与质地应与同期培养物单元菌落基本一致。其主要特点是:菌落大小中等,半透明圆形,向上隆起,表面光滑,边缘整齐,有光泽,无色素产生,用接种针挑取时粘度较大。

然后测定制剂中的活菌数。在干净的环境中称取试样 10.0g 放入装有 90ml 无菌水的灭菌三角瓶中,在旋转式摇床上以 150rpm 充分振荡 1h,选择 3-4 个连续的适宜稀释度,分别用 0.5ml 的无菌吸管吸取 0.1ml 稀释液于已铺好的酵母浸膏培养基平板平板上,每个稀释度至少 5 个重复。 将此稀释度的 5 个平板迅速用无菌玻璃刮刀在琼脂表面均匀涂开,将涂布好后的一系列平板放置于 30℃的培养箱中培养 20~24小时,计数。根据下式统计算出同一稀释度上 5 个平皿上的菌落平均数。

制剂中多粘类芽孢菌含量(CFU/g)=

菌落平均数×总稀释倍数 试样重量

实施例 5 生防菌 HY96-2 对植物青枯病菌的室内毒力测定

在本实施例中,采用的挑战菌株为植物青枯病病原菌为青枯假单孢菌(Ralstonia solanacearum)1号和2号小种Tb和Tt,生防菌株为HY96-2。

抑菌圈测定

用杯碟法测定:将 HY96-2 接菌在 LB 液体培养基中,于转速为 120 转/分钟的 摇床中培养 36 小时,将培养液用离心机高速离心,取上清液用 0.22μm 微孔滤膜(已灭菌)过滤,得无菌滤液。

将青枯病病原菌(*Ralstonia solanacearum*)Tb 和 Tt 分别接菌在 LB 平板上 30℃活化 2 天,用 0.85%生理盐水制成不同浓度梯度菌悬液,用平板计数法确定终菌悬液浓度为 10°cfu/ml,在培养皿中加入 30μl 菌悬液,然后倒入 30ml 45℃的 LB 培养基,摇匀。将 3 只牛津杯放到平板上,分别吸取 200μl 各浓度无菌滤液加入到杯中(或用纸片沾取上述系列浓度无菌滤液,放到平板上)以加无菌水的为对照。每浓度重复 3 次,8℃冰箱中冷冻 4~6 小时,放置在 30℃的恒温箱中培养,18-24 小时后观察结果,测量抑菌直径。结果如下表 5 所示:



表 5	j.	HY96-2	代谢产	物对青	枯病病	原菌的	抑菌效果
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	抑菌圈直径(mm)										
无菌滤液	指示	-	平均								
	菌株	1	2	3	1 20						
	Tb	0	0	0	0						
对照	Tt	0	0	0	0						
	Tb	10.22	9.65	10.25	10.04						
1000 倍稀释液	Tt	8.98	9.82	10.09	9.63						
	Тb	12.62	12.78	13.57	12.99						
100 倍稀释液	Tt	10.88	11.46	11.22	11.19						
	Tb	14.96	16.25	17.78	16.33						
10 倍稀释液	Tt	14.62	15.87	15.59	15.36						
	Tb	24.25	23.68	24.03	23.99						
母液	Tt	22.52	21.68	21.88	22.03						

本研究利用青枯病病原菌的1号和2号生理小种作为指示菌对生防菌株HY96-2 及其发酵液分别进行拮抗实验,研究表明当用含菌体的发酵液进行拮抗实验时,发现随着时间的延长,抑菌圈直径会变大。故分别对菌体和不含菌体的发酵上清液进行拮抗实验,发现发酵清液抑菌效果也非常明显,而活菌体在开始的几天内抑菌圈直径较小,放置一个星期左右后出现较明显的抑菌现象,同时不同浓度的发酵清液产生的抑菌圈大小不同。由这些现象可以初步推测:抑菌现象的产生主要是由于菌体生长代谢会产生一些对青枯病病原菌有抑制作用的活性物质,也就是说,发酵清液中肯定有某些活性物质在起作用,并且活菌体的存在会进一步加强和巩固抑菌效果。

实施例 5 载体的筛选

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比较不同 pH,不同含水量,不同载体对微生物制剂中 HY96-2 菌株存活的影响。HY96-2 在改进的牛肉浸膏培养液中培养 24 小时,离心,取菌体沉淀与不同的 pH 的磷酸缓冲液混合,常温存放 8-96 小时,结果发现 pH6.2~8.0 范围内细菌基本不受影响。在灭菌的稻壳粉和轻质碳酸钙干粉中加入菌体,调制成不同含水量的菌剂,分别在起始和处理 60 天测定活菌数,结果稻壳粉以 7~16%的含水量存活率最高,为91.2%。轻质碳酸钙干粉以 4~6%得含水量存活率最高,为 82.8%。在 pH7.2,有机载体含水量为 7~16%,无机载体含水量 3~6%的条件下,研究了不同载体对 HY96-2 存活的影响。12 个月后检测活菌含量,结果如下表 6 所示。



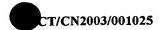


表 6. 载体对 HY96-2 存活的影响

载体	存活率%
稻壳粉	82.9
玉米秸粉	70.5
草炭土	58.9
轻质碳酸钙	60.3
滑石粉	55.6
凹凸棒土	76.3
硅藻土+轻质碳酸钙	62.7

从表 6 中可见,稻壳粉作为载体 HY96-2 存活率最高,是较理想的载体。因此,在下文中选用稻壳粉为制剂载体。由于多粘类芽孢杆菌对干燥耐性较强,因此对含水量要求并不十分严格,为了使多粘类芽孢杆菌在制剂的状态下尽可能保持较高的活性以及较长的贮存期,该微生物制剂的含水量宜在 7-16%之间。

实施例 6 HY96-2 发酵液的直接施用田间防治植物青枯病和增加产量试验

施药方法: 每亩用药量为 2500 毫升 HY96-2 发酵液(1×108CFU/ml)

第一次施药: 浸种与泼浇

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取 100 毫升发酵液,用水稀释 100 倍,然后将用纱布包好的种子(种子量为栽一亩地所需种子)在 HY96-2 稀释液中浸泡 30 分钟,将种子取出阴凉处晾干、播种于苗床上(其面积为栽一亩地所需的苗床,番茄大田定植数以每亩 2500~3000 株左右为准);最后将浸种后的 HY96-2 稀释液均匀泼浇于苗床上。

第二次施药:营养钵假植

移苗或营养钵假植时,取 200 毫升发酵液,加适量清水进行稀释(约为 500~600倍),然后将稀释液均匀泼浇于栽一亩地所需的营养钵中。

第三次施药: 大田定植灌根

大田定植当时,每亩取 1200 毫升发酵液,适量清水进行稀释(约为 500~600 倍), 充然后将稀释液均匀灌根于植株根部。(折合每棵灌药液量 200~250ml 左右)。

第四次施药: 大田定植后 30 天灌根

大田定植后 30 天左右(或始发期),每亩取 1000 毫升发酵液,加适量清水进行稀释(约为 600~700 倍),然后将稀释液均匀灌根于植株根部(折合每棵灌药液量 250~300ml 左右)。

在田间开始发病时调查,在收获期(定植后 80 天)调查发病率,计算防治效果和 25 产量。结果如表 7 所示。

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表 7 大田 HY96-2 发酵菌液对辣椒青枯病防治和增产试验调查结果

调査日期		发	病	ጆ(%)	最后防效	增产率
		定植后	定植后	定植后	(%)	(%)
处理		30 天	50 天	80 天		
	处理	2.0	2.5	15.5	83.5	252.8
番茄	链霉素	33.0	40	67.5	27.9	38.7
	对照 .	55.0	75	93.7		
	处理	1.8	5.2	14.7	82.2	86.8
辣椒	链霉素	10.2	17.5	52.3	36.6	42.5
	对照	19.6	25.2	82.5		
	处理	1.2	3.6	7.0	84.8	75.6
茄子	链霉素	15.3	28.5	30.2	34.3	30.2
	对照	20.3	34.2	46.0		

表结果表明,田间番茄定植后 80 天(收获期),青枯病发病率达到 93.7%,HY96-2 菌液也具有较高的防治效果,防效达 83%以上,增产 252.8%。辣椒田间防效达 82.2%,增产 86.8%。茄子田间防效达 84.8%,增产 75.6%。

实施例 7. 载体形式的微生物制剂温室盆栽试验对番茄青枯病的防治作用和促生长作用

选用番茄感病品种中蔬 6 号,将供试种子播种于蛭石中,待幼苗达 3-4 片真叶时,移栽。

将实施例 4 制得的制剂用水稀释 200 倍,充分搅拌、浸泡 2 小时,再将番茄苗放入浸蘸根部 2 0 分钟,立即移栽于花盆的接菌或未接菌土壤中,并于每钵浇 300 毫升生防制剂。对照用 200 万单位农用链霉素稀释 2000 倍浸根,并设清水处理对照。

青枯菌菌株 Tb(1 号生理小种),在 TZC 培养平板上纯化,于 28-30°C 下在 NA 培养基平板或斜面上培养 48 小时左右,刮取菌苔用灭菌水稀释成浓度为 3×10°CFU/ml 的菌悬液,作为接种体。

细沙壤土 2 份与草炭土 1 份混合均匀,高温消毒,在灭菌土装入盆钵前,估测每盆钵用土重量,称取定量灭菌土,加入适量青枯菌悬液,使土中接种体压力达到 10⁶个细菌/克。将带菌土装入直径 15cm 大小花盆中,同时栽入经生防制剂浸蘸根处理过的番茄苗每盆 2 株,并随即浇制剂 300ml。

在番茄苗移栽后定期调查青枯病发病株数和发病程度(病级),将番茄植株病情按5级划分,以之为基础,计算各处理植株病情指数。在番茄移栽3周后测定上述处理植株的高度、根长、植株和根系的干重或鲜重。

在番茄苗移栽后每10天定期调查青枯病发病株数和发病程度结果如下表8:

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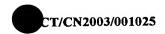


表 8 温室盆栽生物制剂对番茄青枯病的防治作用结果

	移栽		发派	方株 劵	女		发病	发病	病情	病指
处理	后天		折	ラ级				率防	指数	防效
	数	0	1	2	3	4	率(%)	效(%)	(%)	(%)
	10	24	0	0	0	0	0		0	
فالم فالحم والمراز	20	24	0	0	0	0	0	100	0	100
生物制	30	24	0	0	0	0	0	100	0	100
剂	40	22	1	0	1	0	8.3	91.7	4.2	95.5
	50	20	0	1	1	2	16.7	83.3	13.5	86.5
	10	24	0	0	0	0	0		0	
	20	22	1	1	0	0	8.3		3.1	
对照	30	15	0	1	3	5	37.5		42.7	
	40	0	1	1	1	21	100		93.8	
	50	0	0	0	0	24	100		100	
	10	24	0	0	0	0	0		0	
.L. 177 lab	20	23	1	0	0	0	4.2	49.4	1.1	64.5
农用链	30	18	0	0	4	2	25.0	33.3	20.8	51.3
霉素	40	1	0	1	2	20	95.8	4.2	91.7	2.2
	50	0	0	0	0	24	100.0	0	100.0	0

由上表可见,由 HY96-2 制成的生物制剂可将番茄青枯病发生推迟 20 天,当对 照发病率达到 100%时,用药处理才刚刚开始发病,并且病害发生速度缓慢,前期防 效为 95.5%,在后期也具有很高的防治效果,可达 86.5%。而农用链霉素对番茄青枯病的防效很低,到后期已无效果。

番茄在生物制剂处理移栽后,植株生长良好,在前3周内,就表现出生长旺盛的势头,叶片浓绿,叶面积增大,茎杆粗壮,3周后株高明显增加,结果如下表所示。

表 9 生物菌剂对番茄促生长作用结果

L1 755	平	均株高(cn	第6周平均	干重增	
处理	第4周	第5周	第6周	干重(克)	加量(%)
生物制剂	24.6	30.5	38.8	32.8	28.1
对照	14.4	20.5	30.9	25.6	
农用链霉素	14.3	21.2	29.8	25.3	-1.1

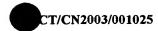
结果表明, HY96-2 的生物制剂对番茄具有明显的促进生长作用, 促生长作用 在前期表现较强, 在 5 周前, 要比对照高 10 厘米以上, 并且茎杆粗壮。在 6 周后,

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用生物制剂处理的植株的干重可增加 28%, 促生长作用显著。

实施例 8. 载体形式的微生物制剂大田试验对植物青枯病的防治作用

在田间用本发明制剂进行防治烟草、番茄、茄子和辣椒青枯病试验,并全面考察生物制剂对植物青枯病的防效和增产效果。经过在田间连续进行三年的防治试验,分别在番茄、辣椒、茄子和烟草等作物上取得了一致稳定的防治效果。下面仅仅列出一年的结果,在不同的发病时期对田间发病情况进行调查,统计最后一次调查(收获后期)的防治效果。

施药方法(每亩用药量为 3000g):

第一次施药: 浸种与泼浇

取 50 克制剂, 用水稀释 300 倍, 充分搅拌 2 小时; 然后将用纱布包好的种子(种子量为栽一亩地所需种子)在稀释液中浸泡 30 分钟, 将种子取出阴凉处晾干、播种于苗床上(其面积为栽一亩地所需的苗床); 最后将浸种后的稀释液均匀泼浇于苗床上。

第二次施药:营养钵假植

移苗或营养钵假植时,取 200 克制剂加适量清水进行稀释(约为 500~600 倍), 充分搅拌 2 小时,然后将稀释液均匀泼浇于栽一亩地所需的营养钵中。

第三次施药:大田定植灌根

大田定植当时,每亩取 1500 克 制剂加适量清水进行稀释(约为 500~600 倍), 20 充分搅拌 2 小时,然后将稀释液均匀灌根于植株根部 (折合每棵灌药液量 200~ 250ml 左右)。

第四次施药: 大田定植后 30 天灌根

大田定植后 30 天左右(或始发期),每亩取 1250 克制剂加适量清水进行稀释(约为 600~700 倍),充分搅拌 2 小时后,然后将稀释液均匀灌根于植株根部(折合每棵灌药液量 250~300ml 左右)。

小区实验处理: 样品 4 个重复,一共 5×4=20 个小区。 结果分别见下表 10-13。





表 10 生物制剂防治番茄青枯病大田试验调查结果

		调	定相	直后 3:	5 天		直后 6	0 天	定	植后 9	0 天	
处理 (g/亩)	重复	査 总 株 数	病株数	病指	防 效 %	病株数	病指	防 效 %	病株数	病指	防 效 %	增 产率 (%)
	1	100	0	0	100	14	2.89	96.09	16	15.07	84.63	l
生物	2	100	0	0	100	10	2.78	96.46	18	16.61	81.87	
制剂	3	100	0	0	100	8	2.22	97.02	20	18.65	80.08	300.83
(3000)	4	100	0	0	100	9	2.30	97.09	19	16.38	83.31	
	X			0	100		2.55	96.67		16.68	82.47	
	1	100	30	3.11	67.06	36	20.56	72.17	59	58.03	40.80	_
链霉	2	100	25	3.22	67.80	35	20.44	73.94	58	57.62	37.10	
素	3	100	19	3.11	70.55	33	20.89	71.98	66	65.68	29.85	53.40
(240)	4	100	25	3.30	64.90	36	20.50	74.05	71	61.35	37.48	
	X			3.19	67.58		20.60	73.04		60.67	36.31	
	1	100	50	9.44	0	95	73.89	0	99	98.03	0	折合亩
ļ	2	100	48	10.00	0	88	78.44	0	94	91.61	0	产量
空白	3	100	55	10.56	0	83	74.56	0	95	93.63	0	721 公
	4	100	50	9.40	0	100	79.00	0	100	98.13	0	
	X		50.8	9.85	0	91.5	76.46	0	97	95.35	0	厅

上表结果表明,生物制剂在番茄田间用量每亩3.0公斤,移栽后60天,防效达96.67%,移栽后90天(收获后期)防效仍为82.47%,增产300.83%,此时对照的发病率高达97%。

表 11 生物制剂防治辣椒青枯病大田试验结果

调查日	定植后 4	0 天	定植后 6	0 天	定植后1	10天	
期	病情指	防治效	病情指	防治效	病情指	防治效	增产率
处 理	数(%)	果(%)	数(%)	果(%)	数(%)	果(%)	(%)
生物制	0	100	0.14	98.43	2.58	79.23	147.9
剂2kg/亩							
链霉素	0.38	74.01	6.33	44.49	7.80	37.08	37.30
0.24kg/亩							
空白	1.47		11.41		12.40		
对照							

上表结果表明,生物菌剂在辣椒田间用量每亩 2.0 公斤,防效达 79.23%,增产 147.9%。

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表 12 生物制剂防治茄子青枯病大田试验结果

调查日	定植后3	0 天	定植后 6	0 天	定植后 9	0 天	增产率
期	病情指	防治效	病情指	防治效	病情指	防治效	(%)
处 理	数(%)	果(%)	数(%)	果(%)	数(%)	果(%)	
3kg/亩	0.11	98.95	1.50	96.43	6.43	85.74	166.6
链霉素	3.75	63.45	9.48	77.39	25.05	44.63	28.80
0.24kg/亩							
空白	10.25		41.93] ——	45.25		
对照							

上表结果表明,生物制剂在茄子田间用量每亩 3.0 公斤,防效达 85.74%,增产 166.6%。

表 13 生物制剂防治烟草青枯病大田试验结果

De 10 = D3.143/14/17 14 11/17								
调查日期	定	植后 110 天						
处 理	发病率(%)	病情指数(%)	防治效果(%)					
3.5kg/亩	52.0	20.9	67.4					
农用链霉素	62.0	26.3	58.9					
空白对照	76.3	64.1						

上表结果表明,在烟草移栽后 110 天,生物制剂田间用量每亩 3.5 公斤,防效达 67%以上。

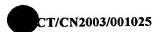
实施例 9. 载体形式的发酵液制剂、活菌制剂、发酵清液制剂对大田番茄青枯病的防治作用

如实施例 4 中 1)、2)、3)所述,将实施例 3 培养的菌株 HY96-2 发酵液的一部分直接配成"发酵液制剂"。对另一部分发酵液进行离心分离,过滤获得活菌,清洗后配成"活菌制剂";另外将分离获得的发酵清液配成"发酵清液制剂"。

田间试验方法与实施例8相同,每亩用药量为2.5千克,结果如下:

表 14 活菌、发酵液和发酵清液配成的制剂防治番茄青枯病病害大田试验结果

调查日期	定植后 40	天	定植后'	70 天	定植后90	天
处 理	病情指数 防 治 效		病情指	防治效果	病情指数	防治效
	(%)	果(%)	数(%)	(%)	(%)	果(%)
发酵液制剂	0	100	0.93	97.68	13.16	83.57
活菌制剂	0	100	1.67	95.83	14.61	81.76
发酵清液制剂	0	100	1.30	96.36	14.15	82.33
农用链霉素	3.52	20.70	17.59	50.78	55.19	31.09
空白对照	4.81		40.00		80.09	





可见,不论是活菌制剂,还是发酵清液制剂对植物青枯病都具有很好的防治效果,并且与发酵液制剂效果相当,无显著差异。

实施例 10. 载体形式的发酵液制剂、活菌制剂、发酵清液制剂对真菌病害的防治 5 作用

在进行实施例 8 的方法时,还发现该微生物制剂对真菌病害也有防治作用。

在安徽省和县的大田试验发现本生物制剂可防治植物苗期立枯病、猝倒病及黄瓜枯萎病,防效达 85%以上。在江西省南昌的大田试验发现本生物制剂对番茄枯萎病和茄子枯萎病具有很好的防治作用,防效达 83%以上。在福建省将乐的大田试验发现其可防治烟草赤星病等真菌性病害,防效达 80%以上。在黑龙江省大田试验发现生物制剂可防治由镰刀菌引起的大豆根腐病病害,防效达 83%以上。结果如下表 15 所示。

表 15 生物制剂防治其它植物真菌病害大田试验调查结果

72.13	-T-100 19713	的相关口性的关键的	古人田瓜业则且纪末
病害名称	制剂种类	发 病 率(%)	防 效(%)
	发酵液	3.2	91.7
亚	活菌	4.8	87.5
番茄立枯病	清液_	4.6	88.1
	清水对照	38.5	
	发酵液	3.8	87.9
775 -++- YA- /70 lui-	活菌	4.2	86.7
番茄猝倒病	清液	4.8	84.8
	清水对照	31.5	
	发酵液	5.15	85.2
وملاس مقالت إلى مقالت ومود	活菌	5.45	84.3
番茄枯萎病	清液	5.82	83.3
	清水对照	34.8	
	发酵液	4.62	85.8
	活菌	5.0	84.6
茄子枯萎病	清液	4.76	85.4
	清水对照	32.5	
	发酵液	2.1	92.7
	活菌	2.6	90.9
黄瓜枯萎病	清液	2.4	91.7
	清水对照	28.8	





病害名称	制剂种类	发	病	率(%)	防 效(%)		
	发酵液		10.5		84.4		
加士士日本	活菌	11.2			83.3		
烟草赤星病 	清液	11.5			82.9		
	清水对照		67.2				
	发酵液		5.3		85.5		
	活菌		5.8		84.2		
大豆根腐病	清液		6.1		83.3		
	清水对照		36.6				

可见,不论是活菌制剂,还是发酵清液制剂对番茄立枯病、番茄猝倒病、番茄 枯萎病、茄子枯萎病、黄瓜枯萎病、烟草赤星病和大豆根腐病都具有很好的防治效 果,并且与发酵液制剂效果相当,无显著差异。

实施例 11. 载体形式的发酵液制剂、活菌制剂、发酵清液制剂对其他植物的促生 长作用

在进行各种生物制剂防治植物青枯病大田试验过程中,发现其对番茄、辣椒、花生等作物生长具有明显的促进作用。生物制剂的大田试验表明:生物制剂不仅对青枯病病原菌寄主作物(如番茄)生长具有明显的促进作用,而且对非青枯病病原菌寄主作物的生长也具有明显的促进作用。试验表明,在番茄无青枯病发生情况下使用本制剂,可使番茄增产高达 27.5%,且番茄的增产效果主要表现为使番茄的前期产量明显增加(见表 16)。其它试验结果表明,应用本生物制剂可使苋菜、菠菜、豇豆和黑麦草分别增产高达 8.3%、25.0%、18.7%和 11.9%(见表 17 和表 18)。

表 16 生物制剂对番茄不发青枯病的增产作用结果

المحادثة المحادثة				采果时间	间和采集	是量(Kg)				۷ ۲۲	4C-2	#\$\$ ~\$\rightar
制剂种	10 月 26	10 月 28	10 月 31	11月3	11月6	11月9	11月12	11月15	11月19		折亩产	増产
类	日	日	日	田	日	日	日	日	日	(Kg)	(Kg)	(%)
对照	0.3	2.0	3.3	0.8	0.6	1.2	1.4	2.2	2.0	13.8	2760	
发酵液	3.3	4.1	3.9	1.0	0.6	1.5	1.2	2.0	0	17.6	3520	27.5
活菌	3.1	3.6	3.5	1.1	0.7	1.3	1.4	2.0	0.2	16.9	3380	22.5
清液	2.8	4.0	4.0	0.8	0.6	1.4	1.2	2.2	0.4	17.4	3480	26.1

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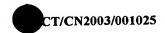


表 17 生物制剂对苋菜、豇豆的增产作用结果

	制剂种类	苋	菜	豆豆						
		株高(cm)	平均株鲜重(g)	株高(cm)	平均株鲜重(g)					
	对照	16.6	6.0	47.3	30.0					
	发酵液	18.1	6.5	52.2	35.6					
	活菌	18.0	6.2	50.8	35.2					
į	清液	17.8	6.5	51.7	35.7					

表 18 生物制剂对菠菜的增产作用结果

					r	
制剂种	平均株高	茎叶鲜重	茎叶干重	根 鲜 重	根干重	增产
类	(cm)	(g)	(g)	(g)	(g)	(%)
对照	12.73	26.59	3.46	0.74	0.15	
发酵液	11.35	33.23	3.70	0.94	0.17	25.0
活菌	12.68	32.56	3.68	1.12	0.16	22.5
清液	12.92	33.18	3.62	0.92	0.17	24.8

根据上述表 16-18 的结果可以知道,不论活菌制剂还是发酵清液制剂对番茄、 苋菜、菠菜、豇豆都具有明显的促生长作用,并且与发酵液制剂无显著差异。

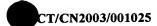
尽管上面已经描述了本发明的具体例子,但是有一点对于本领域技术人员来说 是明显的,即在不脱离本发明的精神和范围的前提下可对本发明作各种变化和改动。 因此,所附权利要求覆盖了所有这些在本发明范围内的变动。

保藏信息

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本发明菌株 HY96-2 已经于 2002 年 10 月 31 日保藏于中国微生物菌种保藏管理委员会普通微生物中心(CGMCC,中国,北京),保藏号为 CGMCC No. 0829。



权利要求

- 1. 一种多粘类芽孢杆菌(Paenibacillus polymyxa) HY96-2, 其保藏号为 CGMCC No. 0829。
 - 2. 一种农用微生物制剂,其特征在于,该微生物制剂含有多粘类芽孢杆菌活菌或利用该菌培养而得的发酵清液。
 - 3. 根据权利要求 2 所述的微生物制剂,其特征在于,该微生物制剂含有利用该菌培养而得的含该活菌及其发酵清液的发酵液。
- 10 4. 根据权利要求 2 所述的微生物制剂,其特征在于,所述微生物制剂还含有选自稻壳粉、玉米秸粉、草炭土、轻质碳酸钙、滑石粉、凹凸棒土和/或硅藻土的载体及其混合物。
 - 5. 根据权利要求 4 所述的微生物制剂,其特征在于,所述载体选自稻壳粉、凹凸棒土或玉米秸粉。
- 15 6. 根据权利要求 5 所述的微生物制剂,其特征在于,所述微生物制剂的含水量在 3-16%(重量)之间。
 - 7. 一种防治植物青枯病的方法,其特征在于,该方法包括将权利要求 2 所述的 微生物制剂施加到患青枯病植物的根部上的步骤。
- 8. 根据权利要求 7 所述的方法, 其特征在于, 所述植物选自番茄、辣椒、茄子 20 和烟草。
 - 9. 权利要求 2 所述的微生物制剂在防治植物苗期立枯病、猝倒病、以及番茄枯萎病、茄子枯萎病、黄瓜枯萎病、烟草赤星病、大豆根腐病上的用途。
 - 10. 权利要求 2 所述的微生物制剂在促进植物生长、提高植物产量上的用途。



PCT

原件(提交) - 打印于 2003年11月28日 (28.11.2003) 星期五 11时23分28秒

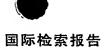
)-1	表格-PCT/RO/134 (EASY) 有关保藏的微生物或其他生物 材料的说明(PCT细则第13条之	
D-1-1	二) 软件版本	PCT-EASY Version 2.92 (更新日期 01.11.2003)
0-2	国际申请号.	
0-3	申请人或代理人的档案号	027229
1	下面的说明与本申请说明书中 此页提到的保藏的微生物或其 他生物材料相关:	
1-1	页码	19
1-2	行号:	12-13
1-3	有关保藏的资料	上层外以上的井北川苏中心
1-3-1	保藏机构名称	中国微生物菌种保藏中心
1-3-2	保藏机构地址	中国微生物菌种保藏委员会, 中国北京市2714信箱, 邮政编码:100080, Beijing (CN)。
1-3-3	保藏日期	2002年10月31日 (31.10.2002)
1-3-4	入藏号	CGMCC 0829
1-4	补充说明	无 (NONE)
1-5	这些说明系对以下指定国作出 :	所有指定国
1-6	单独提交的说明	无 (NONE)
	这些说明将随后提交给国际局	
		由受理局填写
0-4	本表格与国际申请一起收到: (是或否)	
0-4-1	审查官员	印新
		由国际局填写
	国际局收到本表格日期:	
0-5	一四队向议到华农馆日期:	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN03/01025

B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) I IPC7: C12N; A61K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Beloctronic data base consulted during the international search (name of data base and, where practicable, search terms used) GenBank, EMBL,DDBJ,PDB, PIR,WPI C. DOCUMENTS CONSIDERED TO BE RELEVANT Category* Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. X. CN1092397A, SHENYANG APPLIED BIONOMIC INST CHINESE, 2-10 21. Sep. 1.994 (21.09.94), CLAIMS1-4 X. CN1125046A, XINJIANG BIOLOGICAL SOIL DESERT INST CHI, 2-10 26. Jun. 1.996 (26.06.96.), PP6-7 X. CN1074803A, (UVBR-N) UNIV BEIJING, 2-10 04. Aug. 1.993 (04.08.93), PF3-4	A. CLASS	CLASSIFICATION OF SUBJECT MATTER								
Minimum documentation searched (classification system followed by classification symbols) I IPC7: C12N; A61K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Blectronic data base consulted during the international search (name of data base and, where practicable, search terms used) GenBank, EMBL,DDBJ,PDB, PIR,WPI C. DOCUMENTS CONSIDERED TO BE RELEVANT Category* Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. X. CN1092397A, SHENYANG APPLIED BIONOMIC INST CHINESE, 2-10 21.8ep.1994 (21.09.94), CLAIMS1-4 X. CN1125046A, XINJIANG BIOLOGICAL SOIL DESERT INST CHI, 2-10 26.Jun.1996 (26.06.96), PP6-7 X. CN1074803A, (UYBE-N) UNIV BEIJING, 2-10 O4.Aug.1993 (04.08.93), PP3-4 ——————————————————————————————————		IPC7: C12N1/2	0; A61K35/74							
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